

University of Arkansas, Fayetteville ScholarWorks@UARK

Theses and Dissertations

5-2016

Design of a Nutrient Reclamation System for the Cultivation of Microalgae for Biofuel Production and Other Industrial Applications

Heather Sandefur

University of Arkansas, Fayetteville

Follow this and additional works at: <http://scholarworks.uark.edu/etd>

 Part of the [Oil, Gas, and Energy Commons](#), and the [Plant Biology Commons](#)

Recommended Citation

Sandefur, Heather, "Design of a Nutrient Reclamation System for the Cultivation of Microalgae for Biofuel Production and Other Industrial Applications" (2016). *Theses and Dissertations*. 1585.

<http://scholarworks.uark.edu/etd/1585>

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu.

Design of a Nutrient Reclamation System for the Cultivation of Microalgae for
Biofuel Production and Other Industrial Applications

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Chemical Engineering

by

Heather Nicole Sandefur
University of Arkansas
Bachelor of Science in Biological Engineering, 2012

May 2016
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

Dr. Jamie Hestekin
Thesis Co-Director

Dr. Marty Matlock
Thesis Co-Director

Dr. Christa Hestekin
Committee Member

Dr. Robert Beitle
Committee Member

ABSTRACT

Microalgal biomass has been identified as a promising feedstock for a number of industrial applications, including the synthesis of new pharmaceutical and biofuel products. However, there are several economic limitations associated with the scale up of existing algal production processes. Critical economic studies of algae-based industrial processes highlight the high cost of supplying essential nutrients to microalgae cultures. With microalgae cells having relatively high nitrogen contents (4 to 8%), the N fertilizer cost in industrial-scale production is significant. In addition, the disposal of the large volumes of cell residuals that are generated during product extraction stages can pose other economic challenges.

While waste streams can provide a concentrated source of nutrients, concerns about the presence of biological contaminants and the expense of heat treatment pose challenges to processes that use wastewater as a nutrient source in microalgae cultures. The goal of this study was to evaluate the potential application of ultrafiltration technology to aid in the utilization of agricultural wastewater in the cultivation of a high-value microalgae strain. An ultrafiltration system was used to remove inorganic solids and biological contaminants from wastewater taken from a swine farm in Savoy, Arkansas. The permeate from the system was then used as the nutrient source for the cultivation of the marine microalgae *Porphyridium cruentum*.

During the ultrafiltration system operation, little membrane fouling was observed, and permeate fluxes remained relatively constant during both short-term and long-term tests. The complete rejection of *E. coli* and coliforms from the wastewater was also observed, in addition to a 75% reduction in total solids, including inorganic materials. The processed permeate was

shown to have very high concentrations of total nitrogen (695.6 mg L^{-1}) and total phosphorus (69.1 mg L^{-1}).

In addition, the growth of *P. cruentum* was analyzed in a medium containing swine waste permeate, and was compared to *P. cruentum* growth in a control medium. A higher biomass productivity, lipid productivity, and lipid content were observed in the microalgae cultivated in the swine waste medium compared to that of the control medium. These results suggest that, through the use of ultrafiltration technology as an alternative to traditional heat treatment, agricultural wastewaters could be effectively utilized as a nutrient source for microalgae cultivation.

ACKNOWLEDGEMENTS

Support for this project was provided by the University of Arkansas Division of Agriculture, the Ralph E. Martin Department of Chemical Engineering, the Arkansas Biosciences Institute and the Arkansas Water Resources Center. The microalgae cultivation component of this project involved a joint research effort between the author and Maryam Asgharpour, a PhD candidate in the Ralph E. Martin Department of Chemical Engineering. Important contributions to the laboratory work and data collection efforts described in this document were made by Jessica Vaden, Emily Gottberg, and Jason Mariott.

DEDICATION

For Murbella, Dahlia, Max and Madelyn.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION.....	1
1.1. Microalgae Cultivation for Industrial Applications.....	1
1.2. Waste Utilization with Ultrafiltration	1
1.3. Proposed Process and Research Objectives.....	2
CHAPTER 2: LITERATURE REVIEW	5
2.1. Omega-3 Production and Other Industrial Applications for Microalgae.....	5
2.2. Cultivation of Microalgae in Photobioreactors.....	11
2.3. Challenges Associated with Microalgae Cultivation.....	12
2.4. Nutrients in Municipal, Agricultural, and Industrial Waste Streams.....	14
2.5. Ultrafiltration for Nutrient Reclamation from Waste Streams	17
CHAPTER 3: RECOVERY OF NUTRIENTS FROM SWINE WASTEWATER USING ULTRAFILTRATION: APPLICATIONS FOR MICROALGAE CULTIVATION IN PHOTOBIOREACTORS	22
Abstract.....	22
3.1. Introduction.....	23
3.2. Materials and Methods.....	27
3.2.1. Wastewater Feed Source.....	27
3.2.2. Ultrafiltration System Operation.....	27
3.2.3. Feed and Permeate Sampling and Analysis	30
3.2.4. Microalgae Culturing and Analysis	30

3.3.1.	Ultrafiltration Operation	32
3.3.2.	Bacterial and Solids Rejection Performance.....	38
3.3.3.	Microalgae Growth Results	38
3.4.	Conclusions.....	40
	Acknowledgements.....	41
	References.....	42
	CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS.....	45
	WORKS CITED	47
	APPENDIX A: ULTRAFILTRATION SYSTEM CLEANING PROCEDURE	53

LIST OF FIGURES

Figure 1. Experimental design and workplan.	4
Figure 2. Process flow diagram of lipid extraction process. After cultivation in a bioreactor, the microalgae culture is dewatered and pre-treated for lipid extraction and debris removal (Halim et al., 2012).	10
Figure 3. Anaerobic digestion process. Adapted from Qasim (1999).	16
Figure 4. Schematic of a hollow fiber membrane cartridge. Adopted from Wang (1999).	18
Figure 5. Filtration spectrum for common separations processes, membrane sizes, and common contaminants. Adopted from Osmonics, Inc. (2015).	20
Figure 6. Ultrafiltration system setup. Hollow fiber membrane cartridges with a 50,000 molecular weight cutoff were used to remove solids and biological contaminants from the feed.	28
Figure 7. Permeate flux vs. time measured during the short term (2 hour) swine waste challenges. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.	34
Figure 8. Permeate flux vs. time measured during the long-term (8 hour) swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.	35
Figure 9. Permeate flux vs. transmembrane pressure (TMP) measured during the swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.	36

Figure 10. Change in permeate flux with increasing feed concentration measured during the swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.....	37
Figure 11. Biomass productivity and lipid productivity as well as lipid contents from <i>P. cruentum</i> grown in a control medium in addition to swine waste medium.....	39

LIST OF TABLES

Table 1. Nutrient contents of waste from various livestock species. Nutrient data was collected by Garg et al. (2005).	13
Table 2. Example water quality characteristics of the swine waste permeate. The results shown are from the samples collected from ultrafiltration Run #1. The permeate sample was taken after two hours of ultrafiltration operation in recycle mode. The feed was composed of swine wastewater from a holding lagoon. The permeate samples were used as a nutrient source for the cultivation of microalgae.	33
Table 3. Rejection performance of a hollow fiber membrane (50,000 MWCO) operating at 27°C and a transmembrane pressure of 17.5. The feed was composed of a 1:2 dilution of swine wastewater with pure water.	33

LIST OF PUBLISHED PAPERS

Sandefur, H.N., Asgharpour, M., Mariott, J., Gottberg, E., Vaden, J., Matlock, M., Hestekin, J. (2015). Recovery of nutrients from swine wastewater using ultrafiltration: applications for microalgae cultivation in photobioreactors. Ecological Engineering. Under Review.

CHAPTER 1: INTRODUCTION

1.1. Microalgae Cultivation for Industrial Applications

Algal biomass has been identified as a promising feedstock that could be used in a number of industrial applications, including biofuel production, aquaculture, and pharmaceutical production (Sandefur et al., 2014; Jones and Mayfield, 2012; Lam and Lee, 2012; Wang et al., 2013). Through the use of sunlight and CO₂, microalgae species are capable of producing biomass that is rich in lipids and carbohydrates, which can be extracted from the plant material and used in the production of commodities such as biofuels (Cai et al., 2013). Algae have been shown to have higher rates of productivity and higher lipid contents than traditional bioenergy crops, do not require high quality land for cultivation, and would not compete with current agricultural products for space (Sandefur et al., 2011; Wiley et al., 2013). Examples of these high-value algae species include *Nannochloropsis oculata* and *Chlorella vulgaris*, which have been used in the production of biodiesel (Converti et al., 2009), in addition to *Porphyridium cruentum*, which has been identified as a source of omega-3 fatty acids (Ryckebosch et al., 2014).

1.2. Waste Utilization with Ultrafiltration

While the high productivity and lipid content of microalgae make it a promising feedstock for the production of biodiesel and some pharmaceutical products, there are some challenges associated with their use. The production of microalgae can require significant amounts of nitrogen and phosphorus fertilizer (Sialve et al., 2009). As a solution to this problem, agricultural wastes have been identified as an alternative to inorganic fertilizer in microalgae

cultivation (Cai et al., 2013). There have been a number of studies utilizing waste streams in the cultivation of microalgae (Honda et al., 2012; Zhu et al., 2013; Wang and Lan, 2011; Voltolina et al., 2005; Feng et al., 2011). However, there are a number of challenges associated with the use of waste streams as a nutrient source for microalgae production, principally the potential for bioreactor contamination from bacteria (FAO, 2013). In the studies cited above, concerns about contamination were addressed through the preparation of a laboratory-simulated waste, or through pre-treatment with heat for sterilization.

Given the high cost of heat treatment for large volumes of wastewater at the industrial scale, an alternative treatment method will be necessary in order for the use of wastewater as a nutrient source to be feasible in large-scale production. Membrane separations technology is an alternative method for the removal of biological contaminants from the nutrient-rich wastewater prior to use in microalgae cultivation. Microfiltration and ultrafiltration technologies are increasingly common in large-scale municipal waste water treatment operations. In addition, a number of studies have demonstrated the complete rejection of biological contaminants from waste streams using hollow fiber membranes (Teo, 2000; Wang, 1999; Gerardo et al. 2013).

1.3. Proposed Process and Research Objectives

In order to address the issue of nutrient sourcing in microalgae-based industrial applications—and assess the feasibility of utilizing waste streams as a nutrient source—a series of experiments were developed to evaluate the efficacy of hollow fiber membrane technology for removing contaminants from waste streams in a nutrient recapture process. The objectives of this study were to 1) evaluate the potential use of ultrafiltration technology in the removal of inorganic solids and biological contaminants (principally bacteria) from agricultural wastewater

effluent, 2) determine if the treated wastewater was a viable source of nutrients for the production of the high-value microalgae *Porphyridium cruentum*, and 3) determine how resilient *P. cruentum* is to contamination from biological contaminants under optimized growth conditions. The system design and workplan is illustrated in Figure 1.

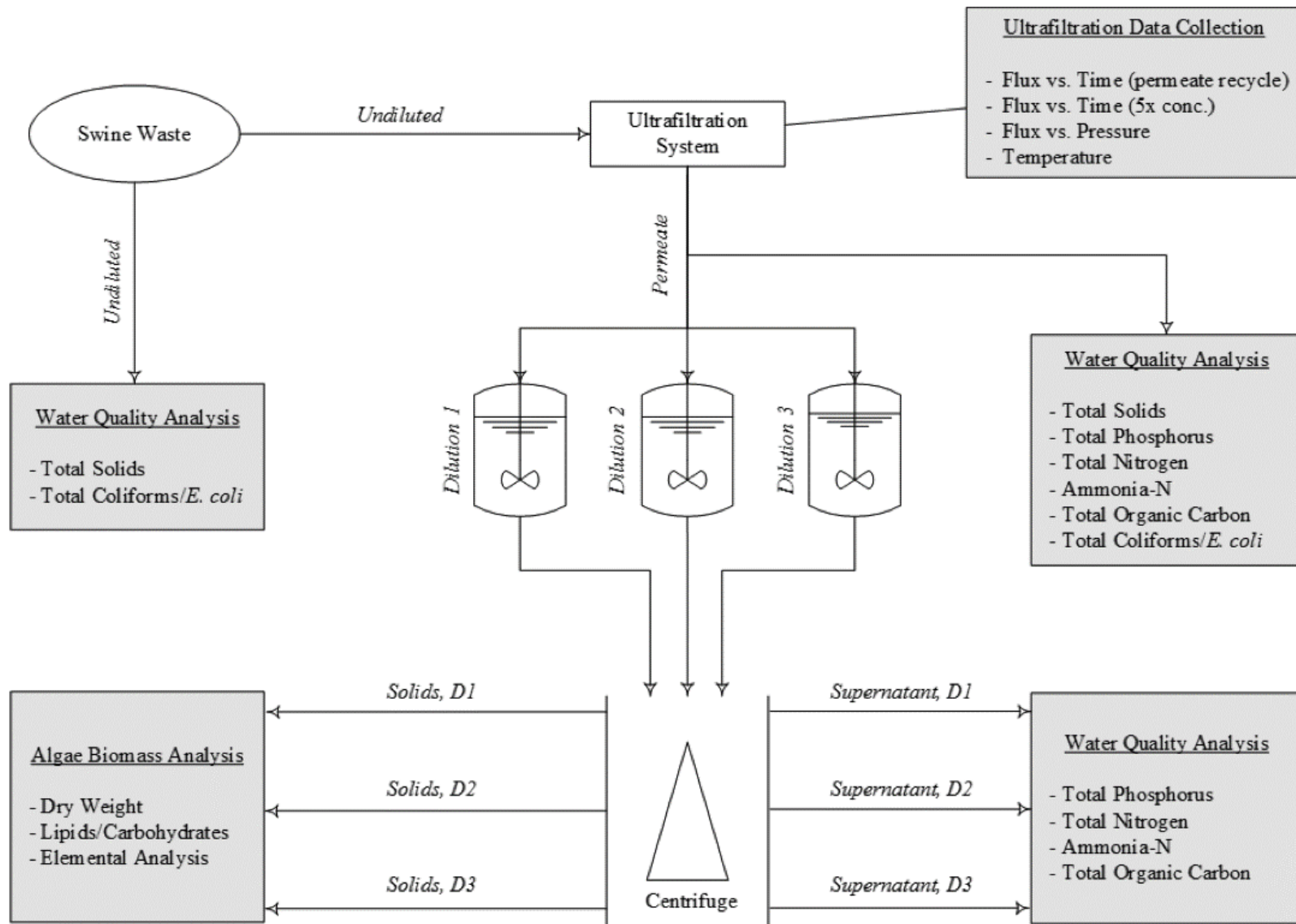


Figure 1. Experimental design and workplan.

CHAPTER 2: LITERATURE REVIEW

2.1. Omega-3 Production and Other Industrial Applications for Microalgae

Algal biomass has been identified as a promising feedstock that could be used in a number of industrial applications, including biofuel production, aquaculture, and pharmaceutical production (Sandefur et al., 2014; Jones and Mayfield, 2012; Lam and Lee, 2012; Wang et al., 2013). Examples of these high-value algae species include *Nannochloropsis oculata* and *Chlorella vulgaris*, which have been used in the production of biodiesel (Converti et al., 2009), in addition to *Porphyridium cruentum*, which has been identified as a potential source of omega-3 fatty acids (Ryckebosch et al., 2014).

Omega-3s are long-chain polyunsaturated fatty acids (PUFAs) and are a critical element of the human diet. There are several key types of dietary PUFAs, including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA). These fatty acids play an important role in neurological development, eye function, and cardiovascular health. The importance of omega-3s in the neurological development of fetuses and small children has also been documented in the literature (Ruxton, 2004).

In spite of the critical importance of omega-3s in the growth and development of humans and other animals, mammalian physiology does not allow for the efficient synthesis of omega-3 fatty acids within the body. As a result, humans and other mammals must obtain these essential nutrients from their diet (Abedi and Sahari, 2014). Given the important dietary role of these fatty acids, a burgeoning market for human dietary supplements that include omega-3s—like EPA—has developed. In addition to the direct human consumption of PUFA-rich material from

supplements and nutraceuticals, livestock producers are also utilizing feeds rich in omega-3s in order to produce animal products with elevated omega-3 contents (Alltech, 2016; Burek et al., 2014).

Historically, dietary supplement manufacturers have relied on fish oil as an industrial source of omega-3s. Fish obtain omega-3s through the consumption of algae, and store the fatty acids in their tissues over the course of their lifetime. This yields omega-3 rich oils that can be extracted from fish for use in dietary supplements. In animal feed, dried and ground fish meal is used as a source of protein and fatty acids in the production of fish, poultry, dairy, beef, and pork products (Burek et al., 2014).

However, these traditional, fish-based sources of omega-3 fatty acids have a number of challenges associated with their use in human and animal supplements, including the presence of carcinogenic compounds and heavy metal contaminants. In addition, fish oils often have undesirable odors and flavors, and can be more unstable than other oil products (Abedi and Sahari, 2014). These contaminant issues can pose a higher risk to pregnant mothers and young children, which represent key target consumers for many commercial omega-3 supplements. As is the case in the human consumption of fish oil, the bioaccumulation of contaminants from fishmeal in animal products also poses health concerns for humans consuming the resulting meat and dairy products (Dorea, 2006). In addition, the widespread use of fish meal in animal agriculture has come under fire in recent years as growing concerns about the environmental impacts of overfishing have led to the public scrutiny of feed sourcing practices within the livestock and aquaculture sectors (Abedi and Sahari, 2014).

Driven largely by growing consumer concerns about dietary health, the global market for omega-3 fatty acids is expanding rapidly, and is projected to grow from approximately \$2.8 billion in 2014 to \$7.5 billion by 2021 (15.2% CAGR) (TMR, 2015). This market includes the commercial production of omega-3s for animal feed, dietary supplements, and pharmaceutical products (Brownlie, 2005).

Much of the growth in the omega-3 fatty acids market has been driven by increases in demand for animal feed. In 2014, approximately 980 million tons of animal feed was produced worldwide. This corresponded to a 12.5% increase in feed production since 2011, which was driven by demand from expanding livestock operations in the United States, Europe, and the developing world (Alltech, 2015). While algae-based omega-3 feed ingredients are relatively new, companies like Alltech, DSM, and Evonik have announced plans to invest in new facilities to produce animal feed products from microalgae (Alltech, 2016; Byrne, 2016). In addition, within the human supplement market, brands like Nordic Naturals® have developed algae-based omega-3 products that are marketed towards pregnant women and individuals with vegan diets (Nordic Naturals, 2016). While DHA-rich microalgae products have emerged in recent years, microalgae-based sources of EPA are lacking (Yaakob et al., 2014). Research has shown that *Porphyridium cruentum*, a fast-growing red marine algae, has relatively high EPA and AA contents compared to other algae species (Yaakob et al., 2014).

In addition to the production of supplements and pharmaceutical products, biodiesel production is an active research area utilizing microalgal biomass for industrial products. As the world's population continues to increase, and more individuals in developing nations gain access to energy resources, the worldwide consumption of fossil fuels continues to expand. In 2008, fossil fuel resources accounted for 88% of the world's total energy consumption, with the largest

individual source coming from oil (35% of total energy consumption). While advances in exploration technologies are likely to allow for a continued supply of fossil fuels in the immediate future, concerns about the availability of these resources long term, and their impact on the environment, have resulted in new research into the production of biofuels (Brennan and Owende, 2009).

Through the use of sunlight and CO₂, photosynthetic organisms are capable of producing carbohydrates and lipids. These molecules can be extracted from the plant material and used in the production of biofuels. Traditional biofuel feedstocks include corn (for the production of ethanol) and soybeans and other oil crops for the production of biodiesel. While these crops have been successfully used to produce biofuels, the use of food crops as a fuel source has been widely debated (Jones and Mayfield, 2012). Algal biomass has been identified as a promising feedstock alternative that could replace food crops in the production of biofuels. Algae have been shown to have higher rates of productivity and higher lipid contents than traditional bioenergy crops. In addition, algal biomass does not require high-quality land for cultivation, and would not compete with current agricultural products for space (Sandefur et al., 2011; Wiley et al., 2013).

The biofuels that can be produced from algae include bioethanol, biodiesel, biohydrogen, and butanol (Lam and Lee, 2012; Jones and Mayfield, 2012; Du et al., 2012; Potts et al., 2011). While algal biomass can be converted into a wide range of biofuel products, certain species are better suited than others for conversion into each fuel type. Where carbohydrates are required, as is the case for the production of bioethanol or butanol, macroalgae tend to be better suited (Cai et al., 2010). Macroalgae, which have a low lignin content, can have high sugar contents in excess of 50% (Jones and Mayfield, 2012). For the production of biodiesel, on the other hand,

microalgae are more desirable, with some species having lipid contents of up to 60% dry weight (Cai et al., 2010; Jones and Mayfield, 2012).

During the biodiesel conversion process, the microalgae must be treated for use as a lipid source (see Figure 2). In order to synthesize biodiesel, the lipids must first be extracted from the biomass. The processing stages for the extraction of lipids from the microalgae include dewatering, pretreatment, and lipid extraction (Greenwell et al., 2010; Halim et al., 2012). After harvesting, the microalgae cells are concentrated to remove excess water during the dewatering step. The concentrated cells can then be pretreated in preparation for lipid extraction. There are a number of pretreatment options that can be utilized depending on the desired products. Existing pretreatment processes include biomass drying and cell disruption. Pretreatment by drying eliminates any water remaining in the concentrate, and the dried biomass can be milled into a fine powder for use in the extraction step (Halim et al., 2012). Cell disruption, on the other hand, involves the rupturing of the concentrated cells in order to release the lipids into solution. Existing methods for cellular disruption include sonication, microwaving, autoclaving, bead milling, and high pressure homogenization (Lee et al., 2010; Halim et al., 2012).

Going into the extraction step, the microalgae biomass can be in the form of a concentrate (no pretreatment), a disrupted concentrate, or a dried powder (Halim et al., 2012). During extraction, a solvent is typically added to the biomass to remove the lipids from the other cellular materials. Two common processes for lipid removal include organic solvent extraction and supercritical fluid extraction (Lam and Lee, 2012; Halim et al., 2012). After the lipids are drawn into the solvent solution, the biomass mixture containing lipids, solvent, cell materials, and water (except in the case of pretreatment by drying), is processed using solid-liquid separations to remove the cell debris from the lipid/solvent solution. If necessary, the lipids can then be

extracted from the solvent using a liquid-liquid separations method (Halim et al., 2012). The extracted lipids can then be used in the production of biodiesel via transesterification with homogeneous or heterogeneous catalysis (Greenwell et al., 2010).

An important requirement for the separations process is that it yields oils that are not contaminated with other components of the microalgal cell, such as chlorophyll, that could negatively impact the conversion to biodiesel (Scott et al., 2010). Any potentially valuable co-products contained in the cellular residue should also be considered. In addition to lipids, crude commodity materials such as proteins and minerals can also be of economic value and should be utilized (Greenwell et al., 2010).

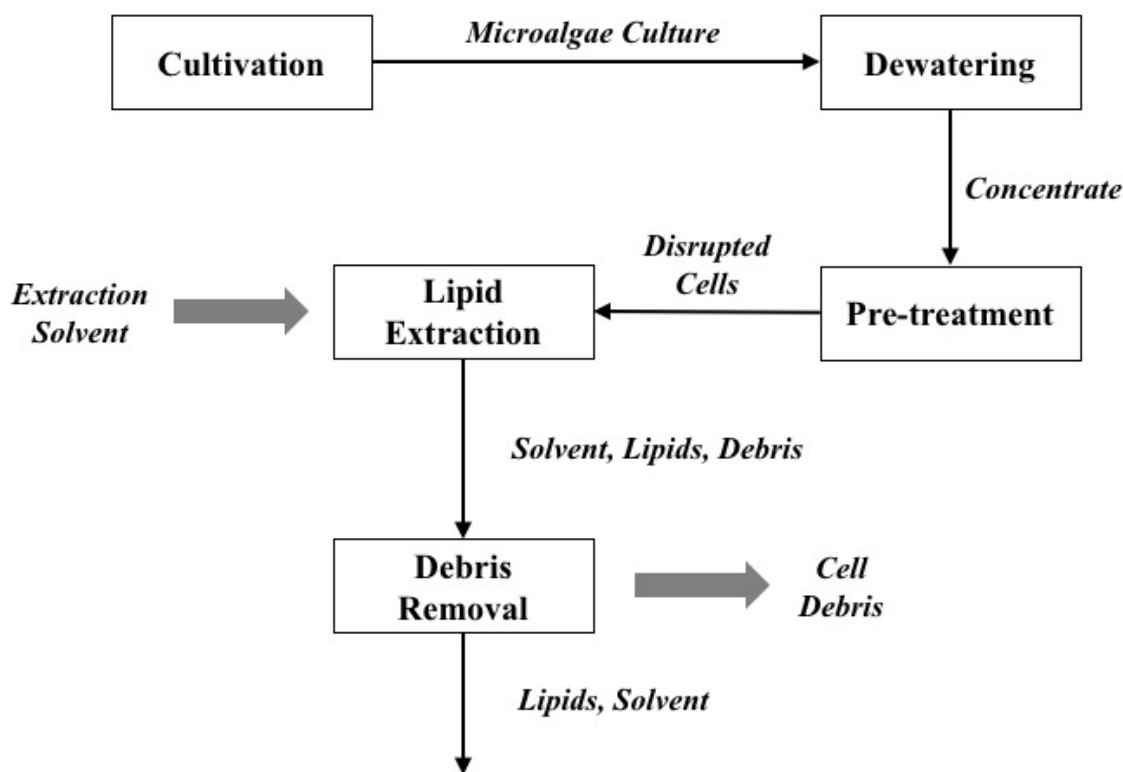


Figure 2. Process flow diagram of lipid extraction process. After cultivation in a bioreactor, the microalgae culture is dewatered and pre-treated for lipid extraction and debris removal (Halim et al., 2012).

2.2. Cultivation of Microalgae in Photobioreactors

Techniques for the cultivation of microalgae include enclosed photobioreactors, open ponds, and attached growth systems (i.e. algal turf scrubbers). Attached growth systems, which involve the cultivation of periphytic macroalgae, can have high growth rates, but are not suitable for the production of microalgae. Photobioreactors and open ponds are the most common systems used in microalgae cultivation (Sandefur et al., 2011). Open ponds consist of small, shallow depressions in which suspended algae grow within the water column. Similar to open ponds, raceway systems are made up of shallow, closed recirculation channels. The simplicity and low operating costs of stirred raceways and unmixed open ponds make them desirable for large scale production (Greenwell et al., 2010). However, because the culture is exposed to the atmosphere in open pond systems, contamination can negatively impact yields. In addition, open pond and raceway systems are not optimized, as the operating parameters are not easily controlled (Greenwell et al., 2010).

Photobioreactor systems are an alternative to open ponds for the cultivation of microalgae. Photobioreactors consist of enclosed growth systems designed and optimized for a particular microalgae culture. Because the systems are enclosed, the operating conditions can be controlled more easily than in pond or raceway systems. In addition, photobioreactors tend to have lower evaporative losses, higher cell densities, and better mixing (Christenson and Sims, 2011). Typical photobioreactors include tubular, flat plate, and bag systems, and are usually operated under phototrophic or photoheterotrophic conditions (Cai et al., 2013; Chen et al., 2011).

The necessary inputs for photobioreactor operation include light, carbon dioxide, and essential nutrients, principally nitrogen and phosphorus. The light source illuminating the reactor can be natural or artificial depending on the reactor location (Supramaniam et al., 2012). There are currently three different sources of carbon dioxide used in the production of microalgae in photobioreactors: air, flue gas, and commercially available carbon dioxide. Many lab-scale photobioreactors use commercially available CO₂; however, issues with cost and availability arise when the systems are scaled up (Rahaman et al., 2011). This is also the case with fertilizer requirements, as large-scale growth systems will require significant nutrient inputs (Sialve et al., 2009). The challenges associated with fertilizer requirements are discussed in depth in the following section.

2.3. Challenges Associated with Microalgae Cultivation

While the high productivity and lipid content of microalgae make it a promising feedstock for biodiesel production, there are some challenges associated with its use. The production of microalgae can require significant amounts of nitrogen and phosphorus fertilizer (Sialve et al., 2009). In order to make the production of microalgae more economical, it is important that low-cost sources of nitrogen and phosphorus are available to producers. Greenwell et al. (2010) reported a nitrogen fertilizer cost of \$1.4 kg⁻¹. Considering the relatively high nitrogen content of microalgae (4 to 8 percent), the nitrogen inputs required for microalgae cultivation can be significant. In addition, the synthesis of nitrogen fertilizer produces around 2 kg of CO₂ kg⁻¹. The additional carbon production, if added to the overall lifecycle, undermines the favorable carbon balance for microalgae feedstocks (Greenwell et al., 2010).

As a solution to this problem, agricultural wastes have been identified as an alternative to inorganic fertilizer in microalgae cultivation (Cai et al., 2013). Wastewater tends to have high amounts of nitrogen and phosphorus (see Table 1), and has been used as a nutrient source for microalgae cultivation in a number of studies (Fenton and hUallachain, 2012; Zhu et al., 2013; Honda et al., 2012). In addition, the problem of nutrient pollution from livestock wastes is a growing challenge for livestock producers. The development of a microalgae-based biological treatment system to replace current nutrient management plans could help to improve the sustainability of livestock production systems (Zhu et al., 2013).

Table 1. Nutrient contents of waste from various livestock species. Nutrient data was collected by Garg et al. (2005).

Waste Type	Moisture Content (%)	Total Organic Carbon (%)	Kjeldahl Nitrogen (%)	C : N Ratio	Total Available Phosphorus (%)
Cow	56.0	47.3	0.53	89.4	0.33
Swine	72.3	51.9	0.56	93.0	0.50
Horse	54.0	48.4	0.35	137.1	0.70
Donkey	54.4	48.5	0.50	97.1	0.50
Sheep	73.4	32.3	0.37	88.9	0.31
Goat	21.8	43.8	0.47	93.5	0.37

Honda et al. (2012) used a simulated treated sewage in the cultivation of the microalgae *Chlorella vulgaris*, *Botryococcus braunii* and *Spirulina platensis* in a flat plate reactor. The reported productivities were comparable to similar studies of microalgae cultivation systems that used traditional nutrient sources. Similarly, Zhu et al. (2013) successfully cultivated the microalgae *Chlorella zofingiensis* in a tubular photobioreactor using swine wastewater as a nutrient source. However, a decrease in lipid content was reported with increasing nutrient concentrations (Zhu et al., 2013).

While the high nutrient concentrations found in animal wastes make them desirable for use in the cultivation of microalgae (Table 1), some problems still exist. Principally, the potential for bioreactor contamination from bacteria is increased when using waste streams as a nutrient source (FAO, 2013). In order to avoid bioreactor contamination, Zhu et al. (2013) sterilized the swine waste in an autoclave prior to use. However, in a large scale system, autoclaving the wastewater prior to use would not be feasible.

2.4. Nutrients in Municipal, Agricultural, and Industrial Waste Streams

The utilization of waste streams as a nutrient source for microalgae production, in addition to generating potential cost savings, can also be used to improve the water quality of impaired freshwater resources. In the United States and other parts of the world, water quality issues associated with eutrophication are widespread. Eutrophication is defined as the enrichment of a water body with high levels of constituent nutrients, most commonly in the form of nitrogen and phosphorus compounds (Dodds and Whiles, 2010). The elevated levels of nitrogen and phosphorus can cause widespread algal blooms that have a rapid period of development followed by senescence, resulting in the production of potentially harmful toxins

and the depletion of dissolved oxygen during decomposition (Dodds and Whiles, 2010). Due to the negative environmental effects on our lakes and rivers, policy makers are placing a high priority on avoiding increased nutrient loading from waste streams including municipal, agricultural, and industrial sources.

Examples of point source emitters for nutrients include municipal wastewater treatment plants, which discharge nutrient-rich wastewater effluent into freshwater systems. In addition, agricultural activities frequently constitute non-point sources for nutrient loads, as rainfall runoff from pastures and manure lagoons can transport the nitrogen and phosphorus from fertilizers and excreta through the watershed (EPA, 2015). By capturing and diverting these nutrients for reuse, an industrial process would have the added benefit of lessening environmental burdens. A number of recent studies have involved the successful cultivation of algae cultures on municipal and agricultural waste streams in order to improve water quality in local watersheds (Sandefur et al., 2012; Sandefur et al., 2014).

There are also a number of nutrient sources contained in industrial waste streams, including the cell residuals that are generated by the lipid extraction stage in the conversion of microalgae to biodiesel. In addition to the nutrient requirements for algae cultivation, the large amounts of residual biomass left over after lipid extraction pose additional economic challenges to the biodiesel production process. In their review of the potential for the use of microalgae as a feedstock for biodiesel, Chisti et al. (2007) suggested that the biomass residuals be anaerobically digested for the production of methane. Anaerobic digestion, as the name implies, involves the use of anaerobic microorganisms to break down organic matter while generating carbon dioxide and methane (Figure 3). Anaerobic digestion is commonly used in wastewater treatment, and in the disposal of organic wastes (Qasim, 1999; Gunaseelan, 1997). The anaerobic digestion

process is generally divided into three steps. In the first step, insoluble organic compounds are converted to soluble organics through hydrolysis. These soluble compounds are then converted into organic acids, and, finally, the volatile organic acids are converted into methane and carbon dioxide (Qasim, 1999).

If coupled with lipid extraction in a biorefinery system, methane production from digestion could make the production of biodiesel from microalgae more economically feasible. Sialve et al. (2009) note the potential for anaerobic digestion to also be used in the recycling of nutrients for microalgae cultivation. By using the anaerobic digestion process to mineralize organic nitrogen present in the residuals into a bioavailable form, it is possible to decrease fertilizer costs while producing a valuable co-product in the form of methane (Sialve et al., 2009).

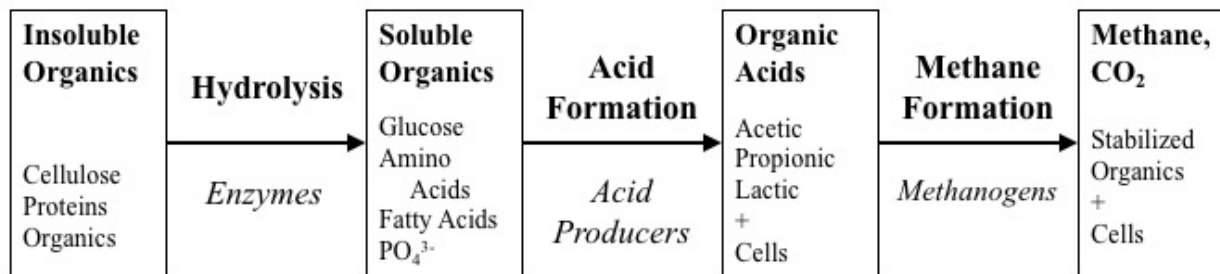


Figure 3. Anaerobic digestion process. Adapted from Qasim (1999).

Each of the nutrient sources described above could be used to supply nitrogen and phosphorus for microalgae production. However, each of these waste streams could potentially contain large amounts of biological contaminants, including high concentrations of bacteria. In order to utilize these waste streams as a nutrient source in a pure culture of microalgae, the waste would need to be processed for contaminant removal.

2.5. Ultrafiltration for Nutrient Reclamation from Waste Streams

At the industrial scale, heat treatment for biological contaminant removal is not economically feasible for large volumes of water. Membrane separations technology constitutes an alternative method for the removal of bacteria from the wastewater prior to use in the photobioreactor. A membrane consists of a thin film that separates two phases, and can be used in liquid-liquid separations. Microfiltration and ultrafiltration involve the use of porous membranes to remove micro- or macro-particles from a solution (Teo, 2000).

The ultrafiltration membrane process lies between nanofiltration and microfiltration in terms of pore size (see Figure 5). The pore sizes for ultrafiltration membranes range from 0.05 μm to 1 μm , and the membranes are commonly synthesized from synthetic polymers or ceramic materials (Mulder, 1996). Ultrafiltration and microfiltration membranes are generally considered to be porous membranes in which the rejection of materials is principally determined by the shapes and sizes of the solutes in relation to the membrane pore size.

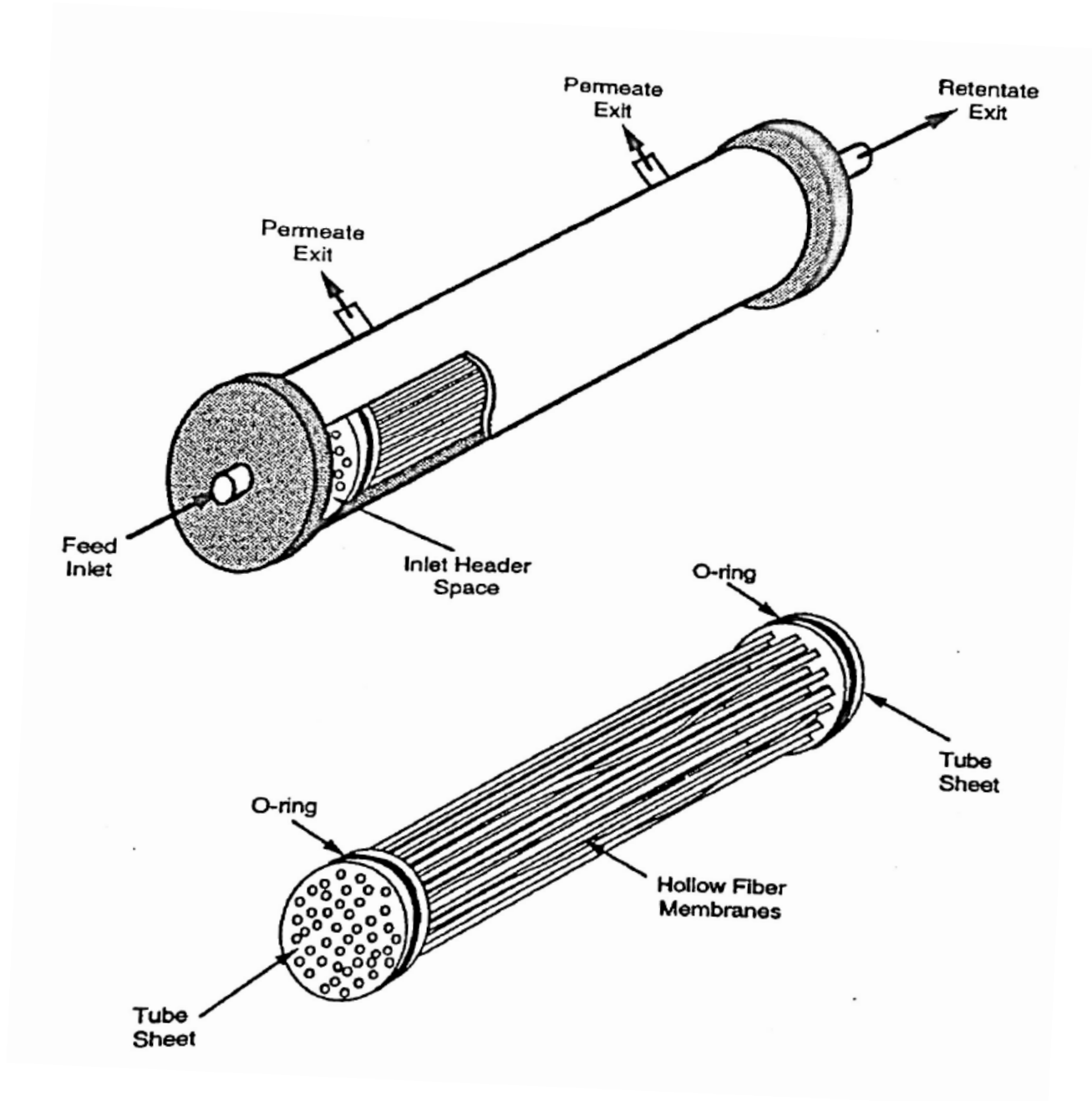


Figure 4. Schematic of a hollow fiber membrane cartridge. Adopted from Wang (1999).

The spectrum of common contaminants compared to membrane pore size in a number of filtration processes is shown in Figure 5. In addition, solvent transport rates are generally considered to be proportional to the applied pressure across the membrane in both ultrafiltration and microfiltration (Mulder, 1996). One key difference between ultrafiltration and microfiltration is related to membrane structure. In ultrafiltration, membranes tend to have an asymmetric structure with a top layer that is much denser than that of membranes used for microfiltration. As a result, ultrafiltration membranes tend to have higher hydrodynamic resistances than membranes used in microfiltration (Mulder, 1996).

Common applications for microfiltration and ultrafiltration include the cold sterilization of pharmaceutical products and beverages, and the clarification of liquids such as alcoholic beverage products (Mulder, 1996). In addition, microfiltration and ultrafiltration have been used in studies for water and wastewater treatment. Both Wang (1999) and Teo (2000) used ultrafiltration in the purification of drinking water, and showed the complete rejection of *E. coli* using a hollow fiber membrane system. Similarly, in their study of nutrient recovery from dairy sludge, Gerardo et al. (2013) used cross-flow microfiltration to obtain a bacteria-free solution while retaining the concentrations of nitrogen and phosphorus.

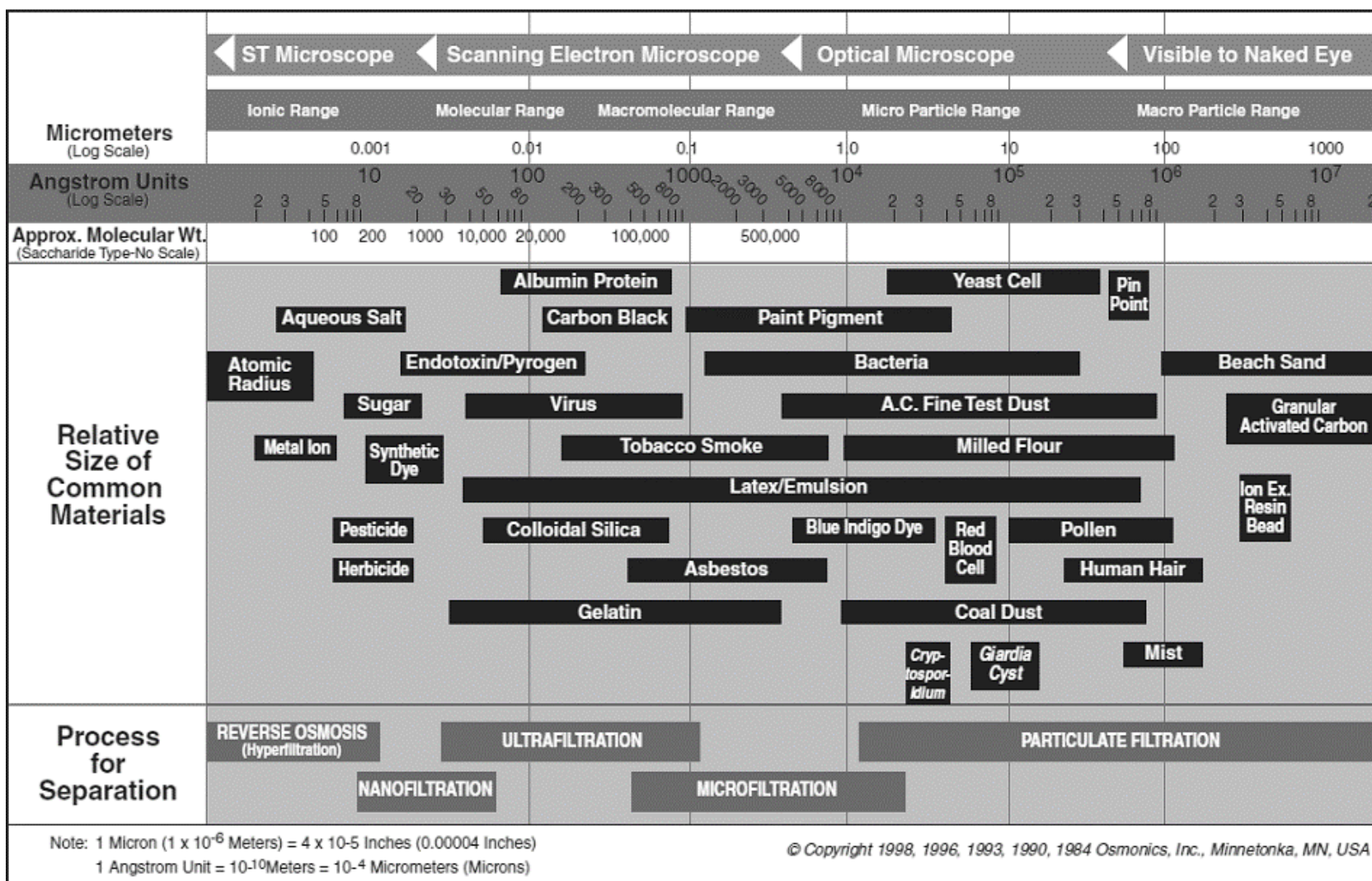


Figure 5. Filtration spectrum for common separations processes, membrane sizes, and common contaminants. Adopted from Osmonics, Inc. (2015).

While the efficacy of microfiltration and ultrafiltration for removing biological contaminants from wastewater has been well established in the literature, raw wastewater poses a challenge to these systems in the form of membrane fouling (Van der Bruggen et al., 2005). Fouling occurs during the ultrafiltration process as a result of the interactions between materials in the feed and the membrane surfaces (Chang et al., 2002). Suspended solids, colloidal particles, biological macromolecules, and plant/animal tissues are commonly found in animal waste and other wastewater streams, and can build up a cake material on the inside of the hollow fiber membranes used during ultrafiltration (Trussell, 2006). The buildup of the cake can cause the membrane permeability to decrease over time, resulting in lower rates of permeate flux within the system (Trussell, 2006).

Consideration of membrane fouling issues is important when utilizing ultrafiltration technology in the treatment of animal wastewater from livestock operations, which often contain high concentrations of the troublesome feed materials described above. There are multiple methods available for removing cake from a membrane in order to counteract fouling, which include bubble aeration (also known as relaxation) in addition to reverse pumping, where the fluid is pumped in the reverse direction across the membrane in order to release the cake buildup (Trussell, 2006). While these methods can be used to effectively reverse many of the effects of cake buildup, fouling remains the primary limitation of membrane filtration systems, and permeate flux must be carefully monitored during operation in order to avoid damage to the membrane materials (Van der Bruggen et al., 2005).

CHAPTER 3: RECOVERY OF NUTRIENTS FROM SWINE WASTEWATER USING ULTRAFILTRATION: APPLICATIONS FOR MICROALGAE CULTIVATION IN PHOTOBIOREACTORS

Submitted for publication in *Ecological Engineering* in December, 2016.

Authors: Heather N. Sandefur, Maryam Asgharpour, Jason Mariott, Emily Gottberg, Jessica Vaden, Marty Matlock, Jamie Hestekin

Abstract

The large-scale production of microalgae poses a number of challenges, including a costly fertilizer demand. While wastewater provides a concentrated source of nutrients, the presence of biological contaminants and the expense of heat treatment are challenging for large-scale production. The goal of this study was to use ultrafiltration to purify wastewater for use in the cultivation of microalgae. Swine waste was filtered, and the resulting permeate was utilized in a *Porphyridium cruentum* culture. Fluxes remained relatively constant during operation, and the complete rejection of bacteria was observed. The permeate contained high concentrations of total nitrogen (695.6 mg L^{-1}) and total phosphorus (69.1 mg L^{-1}). Higher biomass productivity and lipid contents were observed in the microalgae cultivated in the waste medium compared to that of a control medium. This suggests that, by using ultrafiltration as an alternative to heat treatment, agricultural wastewaters could be utilized as a nutrient source for microalgae.

Keywords: ultrafiltration, photobioreactor, swine wastewater

3.1. Introduction

Algal biomass has been identified as a promising feedstock that could be used in a number of industrial applications, including biofuel production, aquaculture, and pharmaceutical production (Sandefur et al., 2014; Jones and Mayfield, 2012; Lam and Lee, 2012; Wang et al., 2013). Through the use of sunlight and CO₂, microalgae species are capable of producing biomass that is rich in lipids and carbohydrates, which can be extracted from the plant material and used in the production of commodities such as biofuels (Cai et al., 2013). Algae have been shown to have higher rates of productivity and higher lipid contents than traditional bioenergy crops, do not require high-quality land for cultivation, and would not compete with current agricultural products for space (Sandefur et al., 2011; Wiley et al., 2013). Examples of these high-value algae species include *Nannochloropsis oculata* and *Chlorella vulgaris*, which have been used in the production of biodiesel (Converti et al., 2009), in addition to *Porphyridium cruentum*, which has been identified as a potential source of omega-3 fatty acids (Ryckebosch et al., 2014).

Omega-3s are long-chain polyunsaturated fatty acids (PUFAs) and are a critical element of the human diet. There are several key types of dietary PUFAs, including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA). These fatty acids play an important role in neurological development, eye function, and cardiovascular health. The importance of omega-3s in the neurological development of fetuses and small children has also been documented in the literature (Ruxton, 2004). However, traditional fish-based sources of omega-3 fatty acids have a number of challenges associated with their use in human and animal supplements, including the presence of carcinogenic compounds and heavy metal contaminants. As a result of these challenges, many pharmaceutical and nutraceutical companies are turning to

microalgae as a source of omega-3 fatty acids. While DHA-rich microalgae products have emerged in recent years, microalgae-based sources of EPA are lacking (Yaakob et al., 2014). Research has shown that *Porphyridium cruentum*, a fast-growing red marine algae, has relatively high EPA and AA contents compared to other algae species (Yaakob et al., 2014; Ryckebosch et al., 2014).

While the high productivity and lipid content of microalgae make it a promising feedstock for a number of industrial applications, there are several challenges associated with their cultivation in large-scale production systems. The production of microalgae can require large amounts of nitrogen and phosphorus fertilizer (Sialve et al., 2009). In order to make the production of microalgae economical, it is important that low cost sources of nitrogen and phosphorus be available to producers. Greenwell et al. (2013) reported a nitrogen fertilizer cost of \$1.40 per kilogram. Considering the relatively high nitrogen content of microalgae (4 to 8%), the nitrogen inputs required for microalgae cultivation can be significant. In addition, the synthesis of nitrogen fertilizer produces around 2 kg of CO₂ kg⁻¹. The additional carbon generation, if added to the overall lifecycle, undermines the favorable carbon balance that is obtained by using microalgae biomass as a feedstock for biofuel production (Greenwell et al., 2013).

As a solution to this problem, agricultural wastes have been identified as an alternative to inorganic fertilizer in microalgae cultivation (Cai et al., 2013). Wastewater tends to have large amounts of nitrogen and phosphorus, and has been used as a nutrient source for microalgae cultivation in a number of studies (Fenton and hUallachain, 2012; Zhu et al., 2013; Honda et al., 2012). In addition, the problem of nutrient pollution from livestock wastes is a growing challenge for livestock producers. The development of a microalgae-based biological treatment

system to replace current nutrient management plans could help to improve the sustainability of livestock production systems (Zhu et al., 2013).

There have been a number of studies utilizing waste streams in the cultivation of microalgae. Honda et al. (2012) used a simulated treated sewage in the cultivation of the microalgae *Chlorella vulgaris*, *Botryococcus braunii* and *Spirulina platensis* in a flat plate reactor. The reported productivities were comparable to similar studies of microalgae cultivation systems that used traditional nutrient sources. Similarly, Zhu et al. (2013) successfully cultivated the microalgae *Chlorella zofingiensis* in a tubular photobioreactor using swine wastewater as a nutrient source.

While the high nutrient concentrations found in animal wastes make them desirable for use in the cultivation of microalgae, some problems still exist. Principally, the potential for bioreactor contamination from bacteria is increased when using waste streams as a nutrient source (FAO, 2013). In their review of contamination pathways for biological pollutants in microalgae cultivation, Wang et al. (2013) noted that the production of microalgal biomass has been historically constrained by biological contamination events that impede production at the industrial scale, even when waste streams are not used. Laboratory-scale studies of microalgae cultivation using waste often involve the preparation of mock waste instead of real wastewater samples (Wang and Lan, 2011; Voltolina et al., 2005; Feng et al., 2011). Studies that utilize authentic wastewater effluent samples have employed a number of treatment methods, including heat treatment and exposure to UV light (Zhu et al., 2013; Cho et al., 2011). For example, in order to avoid bioreactor contamination, Zhu et al. (2013) sterilized the swine waste in an autoclave prior to use. However, in a large-scale system, autoclaving the large volumes of

wastewater prior to use is not economically feasible, and alternative methods for treatment must be developed (Wang et al., 2013).

Membrane separations technology constitutes an alternative method for the removal of biological contaminants from the nutrient-rich wastewater prior to use in microalgae cultivation. A membrane consists of a thin film that separates two phases, and can be used in liquid-liquid separations. Microfiltration and ultrafiltration involve the use of porous membranes to remove micro- or macro-particles from a solution (Teo, 2000). Wang (1999) used ultrafiltration in the purification of drinking water, and showed complete rejection of *E. coli* using a hollow fiber membrane system. Similarly, in their study of nutrient recovery from dairy sludge, Gerardo et al. (2013) used cross-flow microfiltration to obtain a bacteria-free solution while retaining the concentrations of nitrogen and phosphorus, although the use of the recovered nutrients in microalgae cultivation was not explicitly tested (Gerardo et al., 2013).

The objective of this study was to 1) evaluate the potential use of ultrafiltration technology in the removal of inorganic solids and biological contaminants (principally bacteria) from agricultural wastewater effluent, 2) determine if the treated wastewater was a viable source of nutrients for the production of the high-value microalgae *Porphyridium cruentum*, and 3) determine how resilient *P. cruentum* is to contamination from other algae strains under optimized growth conditions.

3.2. Materials and Methods

3.2.1. Wastewater Feed Source

In order to test for the rejection of live cells from an agricultural waste stream, feed samples for the filtration process were obtained from a swine farm located in Savoy, AR (36°6'20"N 94°19'58"W). The feed sample source was an anaerobic digestion lagoon that was used to hold waste flushed from a 150 head grow-finish swine operation, which was used to house weaned pigs until they reach market weight. The facility was made up of a drop curtain style barn that housed pens with fully slatted floors for manure removal via flushing into an adjacent lagoon. For each ultrafiltration run, grab samples of wastewater were taken from the lagoon using a telescopic dipping sampler. The sample was refrigerated after collection for up to 24 hours prior to each ultrafiltration run. Aliquot samples of the wastewater were taken and analyzed to determine the bacteria and total solid concentrations of the feed prior to ultrafiltration.

3.2.2. Ultrafiltration System Operation

The ultrafiltration system used in this study was composed of a feed tank, pump, and 2—1 inch hollow fiber membrane cartridges (50,000 MWCO; Koch Romicon PM50). The pump capacity was 3 gpm at 25 psi. A valve and pressure gauge was located before and after the hollow fiber cartridges in order to control the transmembrane pressure (see Figure 6). Valves 3 and 4 controlled the flow of permeate from the cartridges. The temperature of the fluid in the feed tank was monitored and maintained at 26°C using a chiller unit.

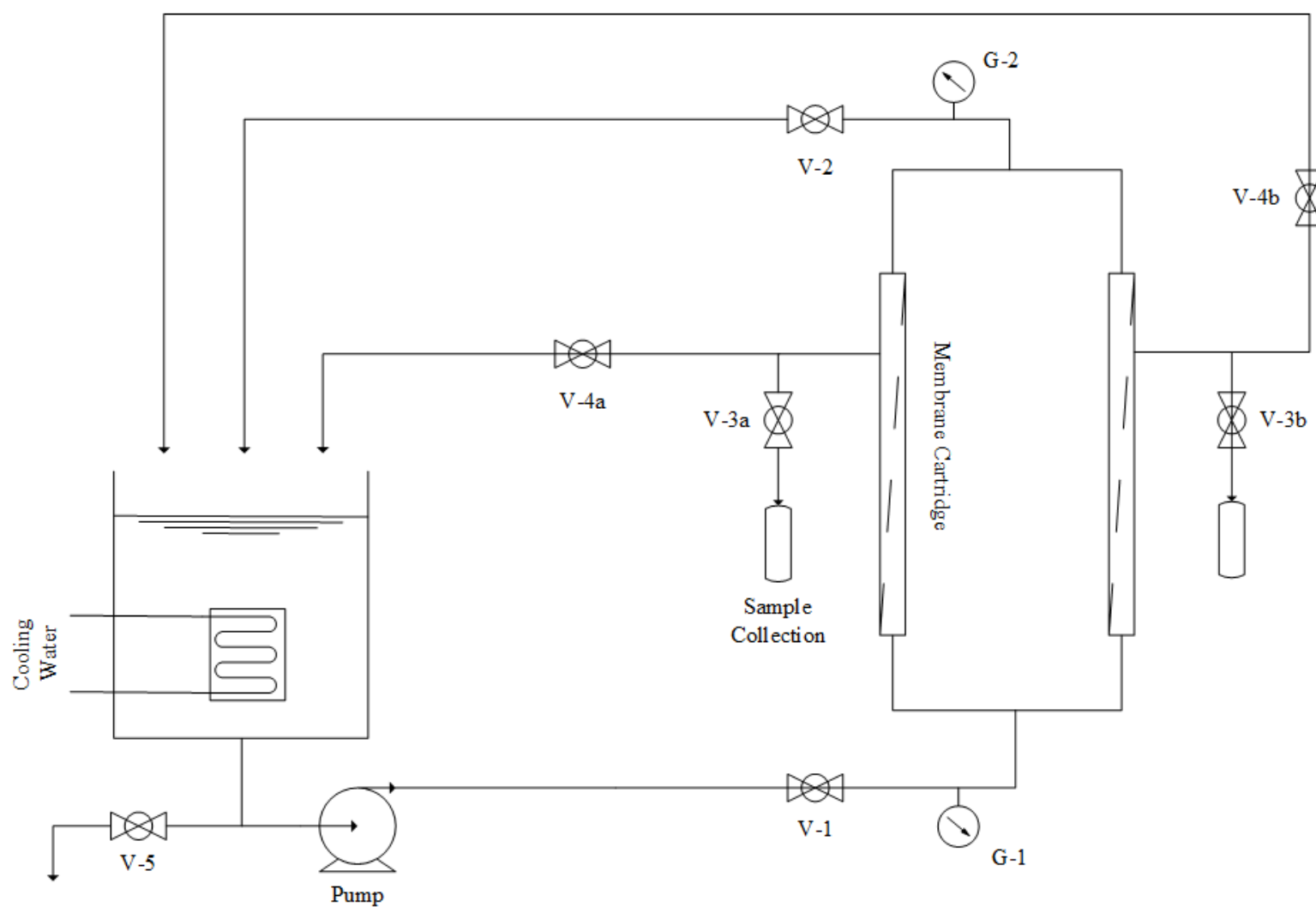


Figure 6. Ultrafiltration system setup. Hollow fiber membrane cartridges with a 50,000 molecular weight cutoff were used to remove solids and biological contaminants from the feed.

To begin the ultrafiltration process, valves V-1 and V-2 were fully closed and opened, respectively (Figure 6). The wastewater feed mixture was then placed in the reservoir and the pump was turned on. V-1 was then slowly opened until 10 psi was read from G-1. V-2 was then closed until a pressure of 15 psi was read from G-2 on the retentate side. V-1 was then adjusted until G-1 read 20 psi, resulting in an average transmembrane pressure of 17.5 psi. Permeate was recirculated through the system for two hours. After one hour, samples were taken from each membrane cartridge via the sampling port by opening valves 3a and 3b. This step was repeated at the two-hour mark.

In addition to the sampling regime, the changes in permeate flux were measured during the ultrafiltration runs. In order to determine if the permeate flux would decrease with each new ultrafiltration cycle, the flux was measured every 10 minutes during three individual two hour runs. The permeate flux was also measured during a long term run, over the course of 8 hours. The flux was determined by measuring the amount of time required to generate 100 mL of permeate, and was normalized against the total membrane area. The values for flux were reported in units of $\text{L m}^{-2} \text{h}^{-1}$, and were plotted over time. In addition, the variations in permeate flux due to changes in transmembrane pressure were also observed. This was achieved by adjusting V-1 and V-2 (see Figure 6) to yield transmembrane pressures ranging from 10 to 25 psi. The clean water permeate flux was also measured before and after each ultrafiltration run to assess any changes in membrane permeability.

Before and after each run, cleaning cycles were performed as recommended by Teo (2000). The cleaning regiment prior to each run was composed of a sanitization cycle made up of a sodium hypochlorite solution, and a rinse cycle with deionized water. After the filtration of swine waste in each run, cleaning cycles were performed in the following order: rinse cycle,

caustic cycle, rinse cycle, sanitization cycle, and rinse cycle. After the cleaning cycles were complete, the hollow fiber cartridges were removed from the ultrafiltration system and stored in a 1% phosphoric acid solution (Teo, 2000).

3.2.3. *Feed and Permeate Sampling and Analysis*

The swine waste feed and permeate were analyzed for bacteria and solids, in addition to nutrient concentration. The number of probable colony forming units of bacteria in each feed and permeate sample was determined using the IDEXX Colilert method (APHA, 2012). The total solids concentration was determined using the EPA 160.3 method (EPA, 2014). In addition to bacteria and total solids, the concentrations of total nitrogen (TN), total phosphorus (TP), ammonia-N, and total organic carbon (TOC) were measured in the feed and permeate samples. Total phosphorus and total nitrogen concentrations were determined using the APHA 4500 PJ method with autoclave digestion (APHA, 2012). Total organic carbon was analyzed using the EPA 351.2 method (EPA, 2014), while ammonia-N concentrations were determined using the APHA 5310 B method with UV digestion (APHA, 2012).

3.2.4. *Microalgae Culturing and Analysis*

The marine microalgae *Porphyridium cruentum* were obtained from the Provasoli-Guillard National Center for Marine Algae and Microbiota (NCMA, W Eel Pond, Woods Hole, Massachusetts, USA). L-1 medium was chosen for culture control and maintenance, and the medium contained the following constituents: NaNO₃, NaH₂PO₄·H₂O, Na₂EDTA·2H₂O, FeCl₃·6H₂O, MnCl₂·4H₂O, ZnSO₄·7H₂O, CoCl₂·6H₂O, CuSO₄·5H₂O, Na₂MoO₄·2H₂O,

H₂SeO₃, NiSO₄·6H₂O, Na₃VO₄, K₂CrO₄, Thiamine-HCl (Vit. B1), Biotin (Vit. H), Cyanocobalamin (Vit. B12), and filtered seawater (Guillard et al., 1993).

Algal cultivation was performed in laboratory-scale sterile corning bottles (VWR). The culture volume for each sample was 100 ml, initially containing approximately 5,000 cells mL⁻¹. Experiments were performed to analyze the growth of *P. cruentum* in swine waste compared to the control medium. Both mediums contained nitrogen concentrations of 82.4 mg L⁻¹ with a salinity of 3.2%, and were kept at optimum environmental conditions (20°C, 140 µE/M².S and a light-dark cycle of 13:11 hours). A more detailed description of the determination of optimum growth conditions will be submitted for publication in a companion article. The swine waste was diluted with seawater and deionized water to the optimum nitrogen concentration and salinity. The biomass was harvested after approximately 18-24 days in the stationary phase using centrifugation. The pellets were lyophilized and then ground into powder prior to fatty acid extraction. A modified method of Bligh and Dyer (1959) was employed to extract lipids from the algal cells.

In addition to the standard cultivation methodology detailed above, the resiliency of *P. cruentum* was assessed by generating replicate cultures, and then intentionally contaminating the cultures. The contamination was achieved by adding 1 mL of raw swine wastewater containing native, freshwater algae species in addition to other biological contaminants. Changes in the growth of the contaminated cultures were observed qualitatively relative to the control replicates.

3.3. Results and Discussion

3.3.1. Ultrafiltration Operation

High levels of total nitrogen, total phosphorus, total organic carbon, and ammonia were observed in the swine wastewater feed samples (see Table 2). Consistently high levels of total solids were also observed for all swine wastewater feed samples (see Table 3), although little change in permeate flux was observed during the ultrafiltration runs. Flux was relatively constant during each of the three 2-hour ultrafiltration runs (see Figure 7); however, an increase in permeate flux was observed from run to run. The difference in flux from experiment to experiment was most likely the result of different concentrations, amounts, and types of solids in the feed stream. Fouling most likely controlled the overall flux and thus we see the slight differences in flux as a result. However, it is important to point out that even with these changes in the membrane there was not a measurable effect on the membrane's bacteria or solids rejection performance (see Table 3).

Table 2. Example water quality characteristics of the swine waste permeate. The results shown are from the samples collected from ultrafiltration Run #1. The permeate sample was taken after two hours of ultrafiltration operation in recycle mode. The feed was composed of swine wastewater from a holding lagoon. The permeate samples were used as a nutrient source for the cultivation of microalgae.

Constituent	Permeate Concentration (mg L ⁻¹)
Total Phosphorus	69.1
Total Nitrogen	695.6
Ammonia	422.8
Total Organic Carbon	598.0

Table 3. Rejection performance of a hollow fiber membrane (50,000 MWCO) operating at 27°C and a transmembrane pressure of 17.5. The feed was composed of a 1:2 dilution of swine wastewater with pure water.

		Coliforms (MPN CFU mL ⁻¹)	E. coli (MPN CFU mL ⁻¹)	Total Solids (mg L ⁻¹)
<i>Run 1</i>				
	Feed	365.4	83.9	4837.0
	Permeate, 1 hr	< 1.0	< 1.0	1200.8
	Permeate, 2 hr	< 1.0	< 1.0	1203.8
<i>Run 2</i>				
	Feed	98.7	3.1	4340.0
	Permeate, 1 hr	< 1.0	< 1.0	1131.4
	Permeate, 2 hr	< 1.0	< 1.0	1117.8
<i>Run 3</i>				
	Feed	12.1	1.7	4599.4
	Permeate, 1 hr	< 1.0	< 1.0	833.3
	Permeate, 2 hr	< 1.0	< 1.0	2812.2

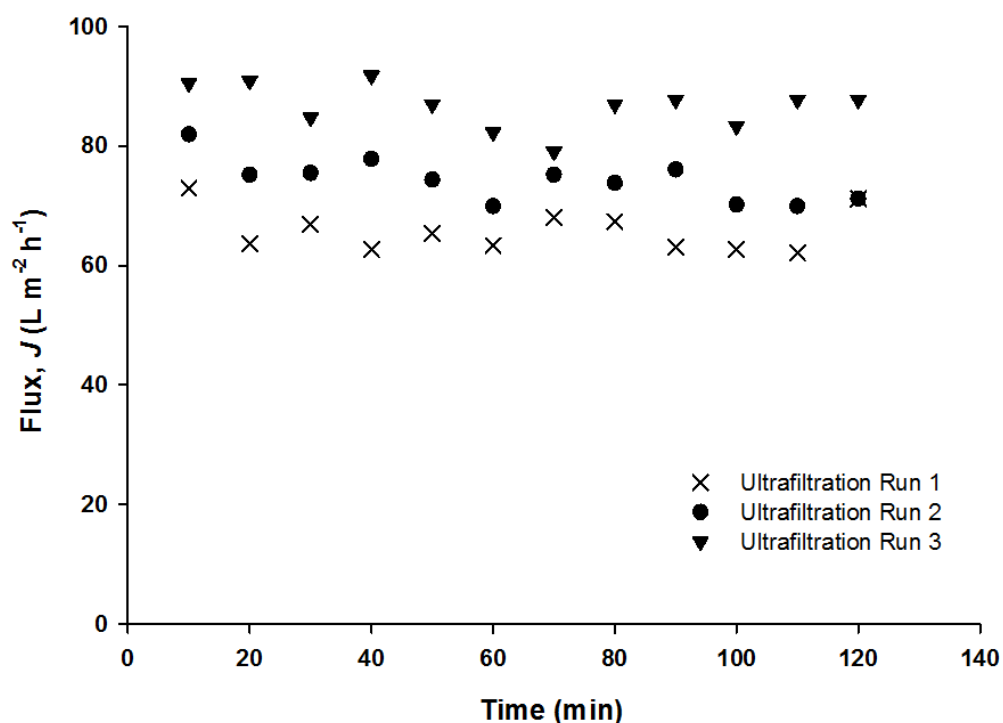


Figure 7. Permeate flux vs. time measured during the short term (2 hour) swine waste challenges. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.

The changes in the rate of permeate flux during the course of the long-term ultrafiltration operation are shown in Figure 8. A relatively small permeate flux decline of approximately 20% was observed over the eight-hour period. Typically, a membrane can operate until the flux decline is more than 80% of the initial flux, suggesting that in this swine waste operation the membrane could operate for a long period of time. Permeate flux rates generally increased with increasing transmembrane pressures, although the increases were somewhat variable (Figure 9). In addition, the results of the concentrate experiment showed a relatively small permeate flux decline of approximately 30% as the feed was concentrated to approximately five times the initial waste concentrations (Figure 10). For each ultrafiltration run, no difference was observed

between the clean water permeate fluxes before and after the swine waste processing. This clearly demonstrates that no permanent degradation of the membrane took place as a result of cleaning or fouling.

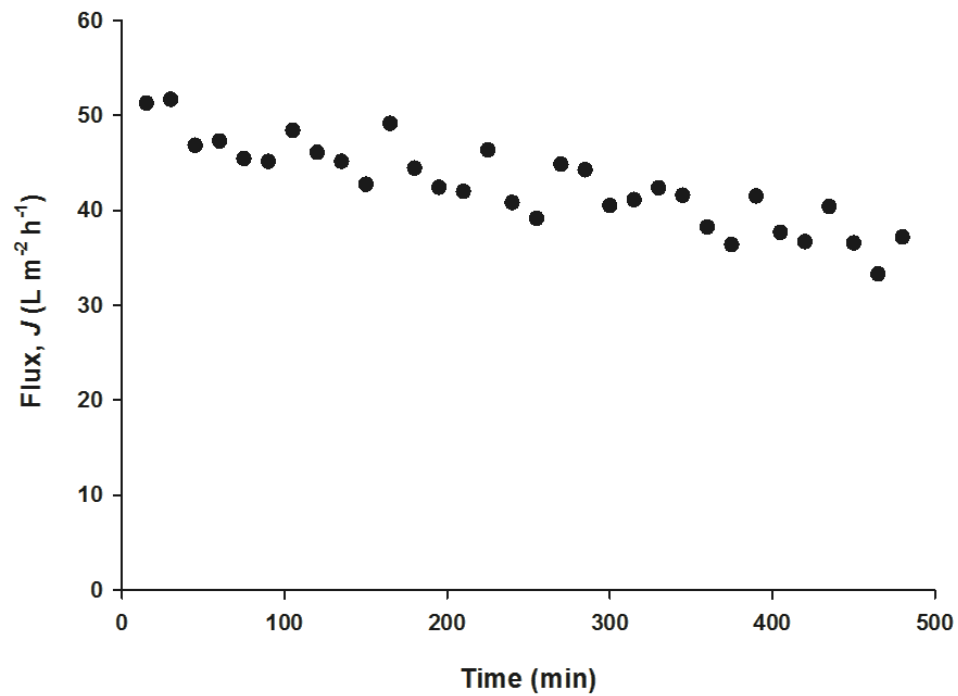


Figure 8. Permeate flux vs. time measured during the long-term (8 hour) swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.

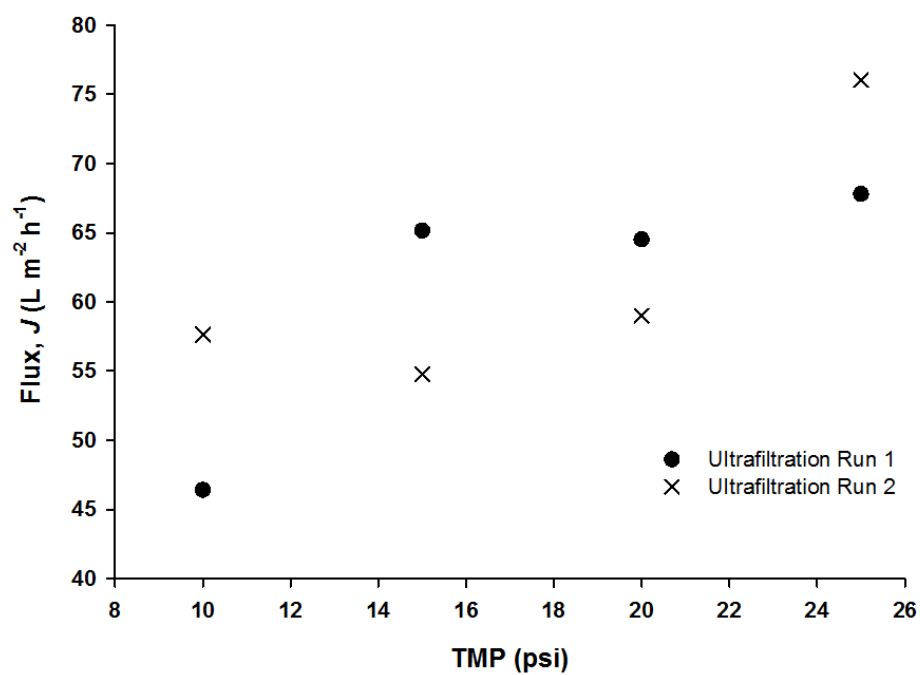


Figure 9. Permeate flux vs. transmembrane pressure (TMP) measured during the swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.

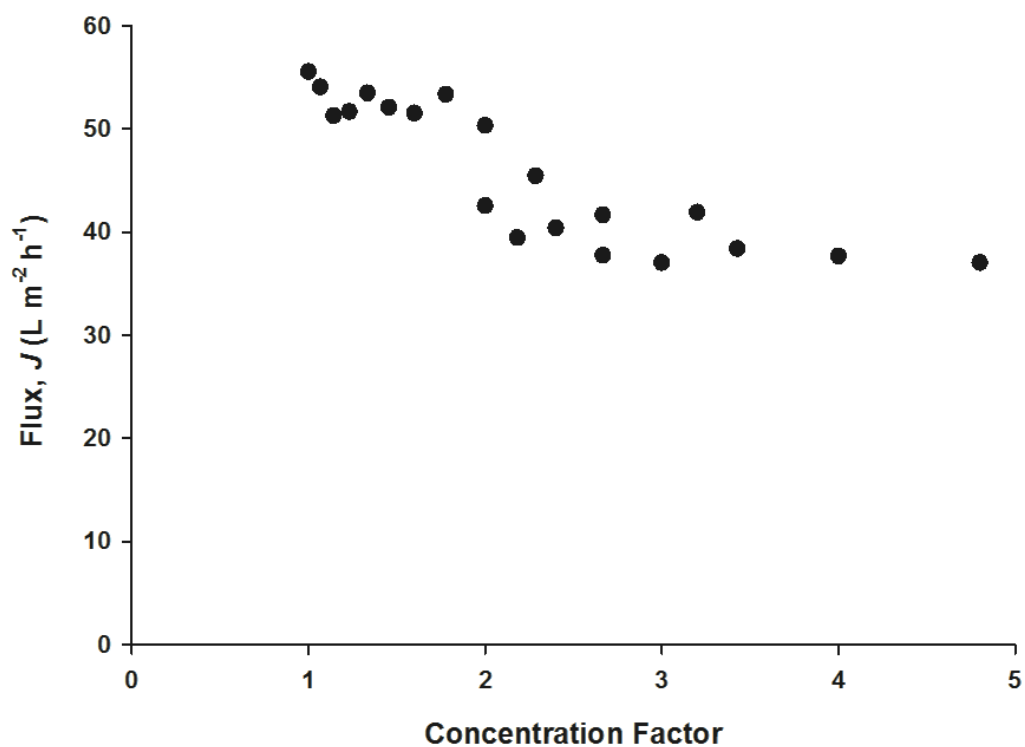


Figure 10. Change in permeate flux with increasing feed concentration measured during the swine waste challenge. In each ultrafiltration run the feed was made up of swine wastewater taken from a storage lagoon.

3.3.2. *Bacterial and Solids Rejection Performance*

As anticipated, the waste feed and processed permeate samples were shown to have high concentrations of total phosphorus, total nitrogen, ammonia-N, and total organic carbon (see Table 2). While significant reductions in nutrient concentrations were observed in the permeate samples compared to the original wastewater feed, constituent concentrations were still much higher than is required for microalgae cultivation. The excess nutrients found in the waste feed were likely bound to the waste solids and were removed during filtration.

In addition to analyzing the nutrient composition of the feed and permeate samples, the concentrations of bacteria and total solids were also measured. While large numbers of coliforms and *E. coli* were found in the waste feed (see Table 3), the amount of bacteria present in the feed was shown to vary, which was likely the result of changing environmental conditions in the wastewater lagoon from which the feed samples were taken. However, the complete removal of coliforms and *E. coli* was observed for each ultrafiltration run (see Table 3). In addition, while the waste feed was shown to have high concentrations of total solids, approximately 75% of the waste solids were consistently removed during the ultrafiltration process (see Table 3).

3.3.3. *Microalgae Growth Results*

The growth of *P. cruentum* was evaluated in the medium containing filtered swine waste compared to the control medium (see Figure 11). The results showed higher biomass and lipid productivity as well as lipid content in the swine waste medium compared to the control medium (Figure 11). Cells tend to accumulate high concentrations of lipids under stress conditions prior to completing the growth process. Generally, the higher biomass and lipid contents of the *P.*

cruentum cells grown in swine waste medium suggest that a process combining ultrafiltration and algae cultivation can be used to effectively convert concentrated swine waste into profitable byproducts while reducing environmental contaminants. However, the observed lipid contents were on the lower end of lipid contents previously reported in the literature, which range from 1% to 14% lipids per unit of dry weight (Asgharpour et al., 2015).

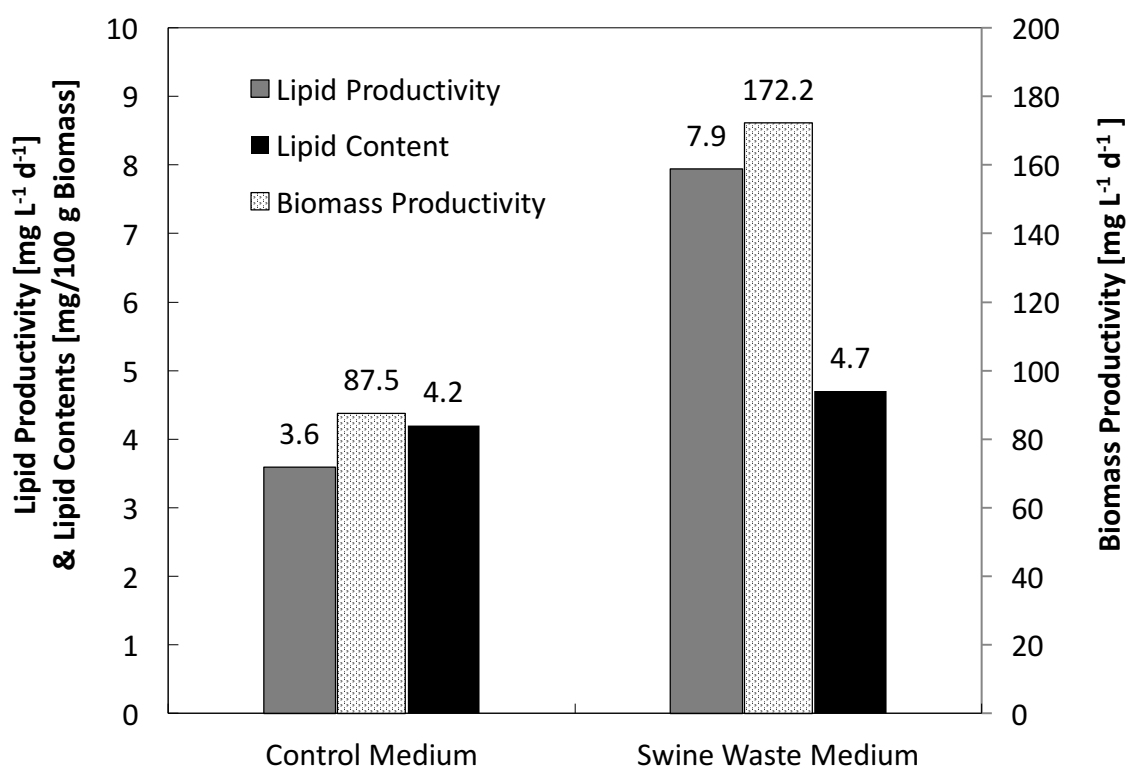


Figure 11. Biomass productivity and lipid productivity as well as lipid contents from *P. cruentum* grown in a control medium in addition to swine waste medium.

The results of the contamination tests showed that, while the contaminated replicates exhibited rapid growth of *P. cruentum* until the last few days of testing, a green algae strain eventually began to dominate the culture, outcompeting *P. cruentum* for nutrients. These results suggest that the saltwater conditions under which the algae was cultivated are not harsh enough to prevent contamination with native algae or bacteria. While *P. cruentum* is a marine algae, these results show that the cultures are still vulnerable to contamination by bacteria and freshwater algae strains.

3.4. Conclusions

The goal of this study was to evaluate the potential application of ultrafiltration technology to aid in the utilization of agricultural wastewater in the cultivation of microalgae. This is the first study of its kind to utilize raw rather than synthetic swine waste to cultivate microalgae while also using ultrafiltration as the treatment mechanism for biological contaminants. The experimental results demonstrated the vulnerability of *P. cruentum* to culture contamination, and illustrate the need for the purification of wastewater streams prior to use as a nutrient source in microalgae cultivation. The results suggest that ultrafiltration could be used as an alternative to heat treatment for the removal of biological contaminants from wastewater. The optimization of microalgae growth using wastewater medium will be explored further in subsequent studies. In addition, a full techno-economic analysis is needed to compare the relative costs of the various mechanisms for contaminant removal.

Acknowledgements

Support for this project was provided by the University of Arkansas Division of Agriculture, the Ralph E. Martin Department of Chemical Engineering, the Arkansas Biosciences Institute and the Arkansas Water Resources Center.

References

- APHA, 2012. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. American Water Works Association, 22nd ed.
- Asgharpour, M., Rodgers, B., Hestekin, J.A. (2015). Eicosapentaenoic Acid from *Porphyridium Cruentum*: Increasing Growth and Productivity of Microalgae for Pharmaceutical Products. *Energies*. 8(9), 10487-10503
- Bligh, E.G., Dyer, W.J, 1959. A rapid method for total lipid extraction and purification. *Can J Biochem Physiol*. 37, 911-917.
- Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*. 19, 360-369.
- Cho, S., Luong, T., Lee, D., Oh, Y., Lee, T., 2011. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. *Bioresource Technology*. 102, 8639-45.
- Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P., Borghi, M.D., 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing: Process Intensification*. 48, 1146-1151.
- EPA, 2014. Analytical Methods and Nominal Quantitation Limits. U.S. Environmental Protection Agency. Retrieved from: <http://water.epa.gov>. Accessed on: 25 April, 2014.
- FAO, 2012. Oilgae: Using Microalgae as a Feedstock for Biodiesel Production. Food and Agriculture Association. United Nations.
- Feng, Y., Li, C., Zhang, D., 2011. Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. *Bioresource Technology*. 102, 101-105.
- Fenton, O., hUallachain, D., 2012. Agricultural nutrient surpluses as potential input sources to grow third generation biomass (microalgae): a review. *Algal Research*. 1, 49-56.

- Gerardo, M.L., Zacharof, M.P., Lovitt, R.W., 2013. Strategies for the recovery of nutrients and metals from anaerobically digested dairy farm sludge using cross-flow microfiltration. *Water Research*. 47, 4833-4842.
- Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J., 2013. Placing microalgae on the biofuels priority list: a review of the technological challenges. *J.R. Soc. Interface*. 7, 703-726.
- Guillard R.R.L., Hargraves, P.E., 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia*. 32, 234-236.
- Honda, R., Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W., Yamamoto, K., 2012. Carbon dioxide capture and nutrients removal utilizing treated sewage by concentrated microalgae cultivation in a membrane photobioreactor. *Bioresource Technology*. 125, 59-64.
- Jones, C.S., Mayfield, S.P., 2012. Algae biofuels: versatility for the future of bioenergy. *Current Opinion in Biotechnology*. 23, 346-351.
- Lam, M.K., Lee, K.T., 2012. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances*. 30, 673-690.
- Rahaman, M., Cheng, L., Xu, X., Zhang, L., Chen, H., 2011. A review of carbon dioxide capture and utilization by membrane integrated microalgal cultivation processes. *Renewable and Sustainable Energy Reviews*. 15, 4002-4012.
- Ruxton, C. (2004). Health benefits of omega-3 fatty acids. *Nursing Standard*. 18:38-42.
- Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K., Foubert, I., 2014. Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. *Food Chemistry*. 160, 393-400.
- Sandefur, H.N., Johnston, R.Z., Matlock, M.D., Costello, T.A., Adey, W.H., Laughinghouse, H.D., 2014. Hydrodynamic regime considerations for the cultivation of periphytic biofilms in two tertiary wastewater treatment systems. *Ecological Engineering*. 71, 527-532.

- Sandefur, H. N., Matlock, M. D., Costello, T. A., Adey, W., Laughinghouse, H. D., 2011. Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecological Engineering*. 37, 1476-1480.
- Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*. 27, 409-416.
- Teo, J.Y., 2000. Rejection of *Escherichia coli* and MS2 bacteriophage by commercially available polysulfone ultrafiltration membrane in drinking water treatment. University of Arkansas, Fayetteville.
- Voltolina, D., Gomez-Villa, H., Correa, G. (2005). Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. *Bioresource Technology*. 96, 359-362.
- Wang, B., Lan, C.Q., 2001. Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent. *Bioresource Technology*. 102, 5639-5644.
- Wang, H., Zhang, W., Chen, L., Wang, J., Liu T., 2013. The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresource Technology*. 128, 745-750.
- Wang, P., 1999. Microfiltration for bacteria and virus rejection in drinking water purification. Thesis, M.S. University of Arkansas, Fayetteville.
- Wiley, P., Harris, L., Reinsch, S., Tozzi, S., Embaye, T., et al., 2013. Microalgae Cultivation Using Offshore Membrane Enclosures for Growing Algae (OMEGA). *Journal of Sustainable Bioenergy Systems*. 3, 18-32.
- Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., & Takriff, M. S. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research*. 21(1):6.
- Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research*. 47, 4294-4302.

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

The goal of this study was to evaluate the potential application of ultrafiltration technology to aid in the utilization of agricultural wastewater in the cultivation of microalgae. This is the first study of its kind to utilize raw rather than synthetic swine waste to cultivate microalgae while also using ultrafiltration as the treatment mechanism for the removal of biological contaminants. The objectives of this study were to 1) evaluate the potential use of ultrafiltration technology in the removal of inorganic solids and biological contaminants (principally bacteria) from agricultural wastewater effluent, 2) determine if the treated wastewater was a viable source of nutrients for the production of the high-value microalgae *Porphyridium cruentum*, and 3) determine how resilient *P. cruentum* is to contamination from biological contaminants under optimized growth conditions.

The experimental results demonstrated that ultrafiltration technology can be utilized to effectively remove biological contaminants from the swine wastewater. In addition, the successful cultivation of *P. cruentum* on the filtered swine wastewater was also demonstrated, with elevated biomass productivities and lipid productivities being observed when compared to the standard, laboratory-prepared medium. The results also demonstrate the vulnerability of *P. cruentum* to culture contamination, and illustrate the need for the purification of wastewater streams prior to use as a nutrient source in microalgae cultivation.

The results suggest that ultrafiltration could be used as an alternative to heat treatment for the removal of biological contaminants from wastewater. While this study focused on swine wastewater as a source of nutrients, the experimental findings suggest that other waste streams could be utilized as nutrient sources in microalgae cultivation. In fact, other nutrient sources—

including municipal and industrial wastewater sources—could be more easily accessible than swine waste for the large-scale production of microalgae in an industrial setting (see Section 2.4).

In order to expand on the knowledge gained in this study, the optimization of microalgae growth using a wastewater medium should be explored further in subsequent studies. In addition, the treatment process for the removal of contaminants from the wastewater should be optimized. In this study, ultrafiltration was selected in consideration of laboratory safety precautions; however, the same levels of bacterial rejection performance could likely be achieved using membranes with higher molecular weight cutoffs (see Figure 5). For a given waste stream, maximum molecular weight cutoffs should be established for bacterial removal in order to optimize the treatment system and minimize the required energy inputs. Improving the feasibility of large-scale microalgae production could support advances in a number of research areas. Society is vitally dependent on advances in the areas of food and energy production, in addition to the development of new pharmaceutical products. Promising applications for microalgae products have been demonstrated in each of these areas, and by attempting to lower the cost of microalgae production, future research efforts in nutrient reclamation could support new developments in each sector.

WORKS CITED

- Trussell, R. S., Merlo, R. P., Hermanowicz, S. W., & Jenkins, D. (2006). The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research*. 40(14):2675-2683.
- Van der Bruggen, B., Cornelis, G., Vandecasteele, C., & Devreese, I. (2005). Fouling of nanofiltration and ultrafiltration membranes applied for wastewater regeneration in the textile industry. *Desalination*. 175(1):111-119.
- Chang, I. S., Le Clech, P., Jefferson, B., & Judd, S. (2002). Membrane fouling in membrane bioreactors for wastewater treatment. *Journal of Environmental Engineering*. 128(11):1018-1029.
- Garg, V.K. (2005). Growth and reproduction of *Eisenia foetida* in various animal wastes during vermicomposting. *Applied Ecology and Environmental Research*. 3:51-59.
- Abedi, E., Sahari, M.A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition*. 2:443-463.
- Alltech. (2015). 2015 Global Feed Survey. Alltech. Nicholasville, KY. Retrieved from: <http://www.alltech.com/sites/default/files/global-feed-survey-2015.pdf>
- Alltech. (2016). Algae – The Growth Platform. Alltech. Retrieved from: www.alltech.com
- APHA. (2012). Standard Methods for the Examination of Water and Wastewater. American Public Health Association. American Water Works Association, 22nd ed.
- Asgharpour, M., Sandefur, H., Vaden, J., Gottberg, E., Hestekin, J. (2016). Fatty acid composition of *Porphyridium cruentum* grown in swine wastewater at multiple concentrations. *Bioresource Technology*. Under Review.
- Baker, R.W. (2012). Membrane Technology and Applications. 3rd Ed. John Wiley and Sons: West Sussex, United Kingdom.
- Beuzenberg, V., Mountfort, D., Holland, P., Shi, F., MacKenzie, L. (2012). Optimization of growth and production of toxins by three dinoflagellates in photobioreactor cultures. *Journal of Applied Phycology*. 24:1023-1033.

- Bligh, E.G., Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Can J Biochem Physiol.* 37:911-917.
- Brennan, L., Owende, P. (2009). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews.* 14(2): 557-577.
- Brownlie, K. (2005). *Functional Food Ingredients. Case Study: The Omega-3 Fatty Acid Market.* Frost and Sullivan.
- Burek, J., Popp, J., Maxwell, C., Ulrich, R. (2014). Developing environmental footprint, cost, and nutrient database of the US animal feed ingredients. *Proceedings of the 9th International Conference on Life Cycle Assessment in the Agri-Food Sector.*
- Byrne, J. (2015). DSM and Evonik in algae derived omega-3 feed joint venture. *FeedNavigator.* Retrieved from: <http://www.feednavigator.com/R-D/DSM-and-Evonik-in-algae-derived-omega-3-feed-joint-venture>
- Cai, T., Park, S.Y., Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews.* 19:360-369.
- Chen, C., Yeh, K., Aisyah, R., Lee, D., Chang, J. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology.* 102:71-81.
- Christenson, L., Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances.* 29:686-702.
- Dodds, W., Whiles, M. (2010). *Freshwater Ecology.* 2nd Edition. Academic Press.
- Dorea, J.G. (2006). Fish Meal in Animal Feed and Human Exposure to Persistent Bioaccumulative and Toxic Substances. *Journal of Food Protection.* 11:2568-2808
- Du, J., McGraw, A., Lorenz, N., Beitle, R.R., Clausen, E.C., Hestekin, J.A. (2012). Continuous fermentation of *Clostridium tyrobutyricum* with partial cell recycle as a long-term strategy for butyric acid production. *Energies.* 5:2835-2848.

- EPA. (2014). Analytical Methods and Nominal Quantitation Limits. U.S. Environmental Protection Agency. Retrieved from:
<http://water.epa.gov/scitech/wastetech/guide/aquaculture/upload/2005_09_01_guide_aquaculture_add_appendixb_508.pdf>. Accessed on: 25 April, 2014.
- EPA. (2015). Agricultural Nonpoint Source Fact Sheet. U.S. Environmental Protection Agency. Retrieved from: <<http://water.epa.gov>>
- FAO. (2012). Oilgae: Using Microalgae as a Feedstock for Biodiesel Production. Food and Agriculture Association. United Nations.
- Feng, Y., Li, C., Zhang, D. (2011). Lipid production of *Chlorella vulgaris* cultured in artificial wastewater medium. *Bioresource Technology*. 102, 101-105.
- Fenton, O., hUallachain, D. (2012). Agricultural nutrient surpluses as potential input sources to grow third generation biomass (microalgae): a review. *Algal Research*. 1(1):49-56.
- Gerardo, M.L., Zacharof, M.P., Lovitt, R.W. (2013). Strategies for the recovery of nutrients and metals from anaerobically digested dairy farm sludge using cross-flow microfiltration. *Water Research*. In Press.
- Greenwell, H.C., Laurens, L.M.L., Shields, R.J., Lovitt, R.W., Flynn, K.J. (2013). Placing microalgae on the biofuels priority list: a review of the technological challenges. *J.R. Soc. Interface*. 7:703-726.
- Guillard R.R.L., Hargraves, P.E. (1993). *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia*. 32:234-236.
- Halim, R., Danquah, M.K., Webley, P.A. (2012). Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology Advances*. 30:709-732.
- Honda, R., Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W., Yamamoto, K. (2012). Carbon dioxide capture and nutrients removal utilizing treated sewage by concentrated microalgae cultivation in a membrane photobioreactor. *Bioresource Technology*. 125:59-64.
- Jones, C.S., Mayfield, S.P. (2012). Algae biofuels: versatility for the future of bioenergy. *Current Opinion in Biotechnology*. 23:346-351.

- Kaiser, G.E. (2011). Lab 4: Enumeration of Microorganisms. Retrieved from: <http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab4/lab4.html>. Accessed: 22 November 2013
- Lam, M.K., Lee, K.T. (2012) Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnology Advances*. 30:673-690.
- Lee, J., Yoo, C., Jun, S., Ahn, C., Oh, H. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresource Technology*. 101:S75-S77.
- Mulder, M. (1996). *Basic Principles of Membrane Technology*. 2nd Edition. Springer, New York.
- Nordic Naturals. (2016). Algae Omega. Nordic Naturals. Retrieved from: www.nordicnaturals.com
- Osmonics. (2015). Filtration Spectrum. Osmonics, Inc. Minnetonka, MN. Retrieved from: <http://www.sswm.info/sites/default/files/toolbox/RADCLIFF%202004%20Filtration%20Spectrum.png>
- Potts, T., Du, J., Paul, M., May, P., Beitle, R., Hestekin, J. (2011). The production of butanol from Jamaica Bay macroalgae. *Environmental Progress and Sustainable Energy*. 31(1): 29-36.
- Qasim, S.R. (1999). *Wastewater Treatment Plants: Planning, Design, and Operation*. 2nd ed. CRC Press: Boca Raton.
- Rahaman, M., Cheng, L., Xu, X., Zhang, L., Chen, H. (2011). A review of carbon dioxide capture and utilization by membrane integrated microalgal cultivation processes. *Renewable and Sustainable Energy Reviews*. 15:4002-4012.
- Razaghi, A., Godhe, A., Albers, E. (2014). Effects of nitrogen on growth and carbohydrate formation in *Porphyridium cruentum*. *Central European Journal of Biology*. 9:156-162.
- Ruxton, C. (2004). Health benefits of omega-3 fatty acids. *Nursing Standard*. 18:38-42.
- Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K., Foubert, I. (2014). Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. *Food Chemistry*. 160, 393-400.

- Sahu, A.K., Siljudalen, J., Trydal, T., Rusten, B. (2013). Utilization of wastewater nutrients for microalgae growth for anaerobic co-digestion. *Journal of Environmental Management*. 122:113-120.
- Sandefur, H. N., Matlock, M. D., Costello, T. A., Adey, W., Laughinghouse, H. D. (2011). Seasonal productivity of a periphytic algal community for biofuel feedstock generation and nutrient treatment. *Ecological Engineering*. 37:1476-1480.
- Sandefur, H., Asgharpour, M., Mariott, J., Gottberg, E., Vaden, J., Matlock, M., Hestekin, J. (2016). Recovery of nutrients from swine wastewater using ultrafiltration: applications for microalgae cultivation in photobioreactors. *Ecological Engineering*. Under Review.
- Sandefur, H.N., Johnston, R.Z., Matlock, M., Adey, W., Costello, T., Laughinghouse IV, H. (2014). Hydrodynamic regime considerations for the cultivation of algae in two tertiary wastewater treatment systems. *Ecological Engineering*. 71:527-532.
- Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., Smith, A.G. (2010). Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology*. 21:277-286.
- Sialve, B., Bernet, N., Bernard, O. (2009). Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances*. 27(4):409-416.
- Supramaniam, J., Palanisamy, K., Nomanbhay, S.M. (2012). Study on the pH changes in microalgae (*Tetraselmis Chuii*) cultivated in newly developed closed photobioreactor using natural sunlight and artificial light. *Journal of Energy and Environment*. 4(1):18-20.
- Teo, J.Y. (2000). Rejection of *Escherichia coli* and MS2 bacteriophage by commercially available polysulfone ultrafiltration membrane in drinking water treatment. Thesis, M.S. University of Arkansas, Fayetteville.
- TMR. (2015). Omega 3 Ingredients Market By Application - Global Industry Analysis, Size, Share, Growth, Trends, and Forecast. Transparency Market Research.
- Voltolina, D., Gomez-Villa, H., Correa, G. (2005). Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. *Bioresource Technology*. 96, 359-362.
- Wang, B., Lan, C.Q. (2001). Biomass production and nitrogen and phosphorus removal by the green alga *Neochloris oleoabundans* in simulated wastewater and secondary municipal wastewater effluent. *Bioresource Technology*. 102, 5639-5644.

- Wang, H., Zhang, W., Chen, L., Wang, J., Liu T. (2013). The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresource Technology*. 128, 745-750.
- Wang, J., Chen, B., Rao, X., Huang, J., Li, M. (2007). Optimization of culturing conditions of *Porphyridium cruentum* using uniform design. *World J Microbiol Biotechnol*. 23:1345-1350.
- Wang, P. (1999). Microfiltration for bacteria and virus rejection in drinking water purification. Thesis, M.S. University of Arkansas, Fayetteville.
- Wiley, P., Harris, L., Reinsch, S., Tozzi, S., Embaye, T., et al. (2013). Microalgae Cultivation Using Offshore Membrane Enclosures for Growing Algae (OMEGA). *Journal of Sustainable Bioenergy Systems*. 3:18-32.
- Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., & Takriff, M. S. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research*. 21(1):6.
- Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research*. 47(13): 4294-4302.

APPENDIX A: ULTRAFILTRATION SYSTEM CLEANING PROCEDURE

The following cleaning cycles were used before and/or after the operation of the ultrafiltration unit (see Section 3.2). The steps in each cleaning cycle are listed below. The cleaning methods used in each cycle were adopted from Teo (2000) and Wang (1999). See Section 3.2 for system component references.

Rinse Cycle:

The rinse cycle was used to flush the system before and/or after each chemical cycle, and removed any residual feed or cleaning solution.

1. Add 7 L of MilliQ water to reservoir.
2. Open valves V-2, V-4a, and V-4b
3. Turn on pump, and gradually open V-1 until gauge G-1 reads 10 psi.
4. Gradually close V-2 until gauge G-2 reads 15 psi.
5. Adjust V-1 and V-2 until G-1 reads 20 psi and G-2 reads 15 psi.
6. Continue running for 10 minutes.
7. Open valves V-3a and V-3b, close V-4a and V-4b. Continue running until 1 L of permeate is drained.
8. Open V-4a and V-4b, close V-3a and V-3b.
9. Return permeate to reservoir and continue operation for 20 minutes.
10. Close V-1 until G-1 reads 10 psi.
11. Turn off pump and open V-5 to drain system.

Sanitization Cycle:

This cycle involved the use of a bleach solution to kill any cell contaminants present in the system lines or hollow fiber cartridges.

1. Prepare 7 L of a 200 ppm sodium hypochlorite (bleach) solution and pour into the feed reservoir.
2. Open valves V-2, V-4a, and V-4b
3. Turn on pump, and gradually open V-1 until gauge G-1 reads 10 psi.
4. Gradually close V-2 until gauge G-2 reads 15 psi.
5. Adjust V-1 and V-2 until G-1 reads 20 psi and G-2 reads 15 psi.
6. Continue running for 10 minutes.
7. Open valves V-3a and V-3b, close V-4a and V-4b. Continue running until 1 L of permeate is drained.
8. Open V-4a and V-4b, close V-3a and V-3b.
9. Return permeate to reservoir and continue operation for 20 minutes.
10. Close V-1 until G-1 reads 10 psi.
11. Turn off pump and open V-5 to drain system.

Caustic Cycle:

The caustic cycle used sodium hydroxide to remove any remaining protein residue in the ultrafiltration system.

1. Prepare 7 L of a 1% sodium hydroxide solution and pour into the feed reservoir
2. Open valves V-2, V-4a, and V-4b

3. Turn on pump, and gradually open V-1 until gauge G-1 reads 10 psi.
4. Gradually close V-2 until gauge G-2 reads 15 psi.
5. Adjust V-1 and V-2 until G-1 reads 20 psi and G-2 reads 15 psi.
6. Continue running for 10 minutes.
7. Open valves V-3a and V-3b, close V-4a and V-4b. Continue running until 1 L of permeate is drained.
8. Open V-4a and V-4b, close V-3a and V-3b.
9. Return permeate to reservoir and continue operation for 20 minutes.
10. Close V-1 until G-1 reads 10 psi.
11. Turn off pump and open V-5 to drain system.

Acid Cycle:

The acid cycle was used occasionally to remove mineral salts from the system. When run, this cycle took place before the caustic cycle.

1. Prepare 7 L of phosphoric acid solution (pH 2-3) and pour into the feed reservoir.
2. Open valves V-2, V-4a, and V-4b
3. Turn on pump, and gradually open V-1 until gauge G-1 reads 10 psi.
4. Gradually close V-2 until gauge G-2 reads 15 psi.
5. Adjust V-1 and V-2 until G-1 reads 20 psi and G-2 reads 15 psi.
6. Continue running for 10 minutes.
7. Open valves V-3a and V-3b, close V-4a and V-4b. Continue running until 1 L of permeate is drained.
8. Open V-4a and V-4b, close V-3a and V-3b.

9. Return permeate to reservoir and continue operation for 20 minutes.
10. Close V-1 until G-1 reads 10 psi.
11. Turn off pump and open V-5 to drain system.



February 17, 2014

MEMORANDUM

TO: Dr. Jamie Hestekin

FROM: W. Roy Penney
Institutional BioSafety Committee

RE: IBC Protocol Approval

IBC Protocol #: 14022

Protocol Title: "Purification of Agricultural Wastewater for the Cultivation of Microalgae"

Approved Project Period: Start Date: February 13, 2014
Expiration Date: February 12, 2017

The Institutional Biosafety Committee (IBC) has approved Protocol 14022, "Purification of Agricultural Wastewater for the Cultivation of Microalgae" You may begin your study.

If further modifications are made to the protocol during the study, please submit a written request to the IBC for review and approval before initiating any changes.

The IBC appreciates your assistance and cooperation in complying with University and Federal guidelines for research involving hazardous biological materials.